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Journal of Environmental Management 84 (2007) 93–99

Journal of Environmental Management

www.elsevier.com/locate/jenvman

Beneficial effect of saprobe and arbuscular mycorrhizal fungi on growth of *Eucalyptus globulus* co-cultured with *Glycine max* in soil contaminated with heavy metals

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Available online 11 July 2006

Abstract

The effects of saprobe and arbuscular mycorrhizal (AM) fungi on growth, chlorophyll and N, P and K content of *Eucalyptus globulus* Labill. growing in soil contaminated by heavy metals in the presence or absence of *Glycine max* were investigated. *Glomus mosseae* and *Glomus deserticola* increased dry weight, shoot length, total N, P and K concentration and the quantity of chlorophyll in *E. globulus* shoots. The protection of *Eucalyptus* by AM fungi against the action of the heavy metals was more evident when this plant grew as an intercrop with soybean than as a monoculture. The presence of the saprobe fungi *Fusarium concolor* and *Trichoderma koningii* further enhanced shoot dry weight, N, P and K content of AM *Eucalyptus*. The co-inoculation of *Eucalyptus* with *Glomus deserticola* and *T. koningii* was more effective for Cd uptake. In addition, *Glomus deserticola* enhanced the amount of Pb absorbed by *Eucalyptus* plants. We showed that it is important to select the most efficient AM and saprobe fungi to stimulate plant growth in heavy-metal-contaminated soil and that the combination of both plays an important role in metal tolerance of *Eucalyptus* plants.

Keywords: Eucalyptus; Contamination; Heavy Metals; Arbuscular mycorrhiza; Saprobe fungi; Cadmium; Lead; Tolerance

1. Introduction

Heavy metal contamination of soil due to industrialization and other human activities has become an environmental problem with consequent problems for the human population. High concentrations of heavy metals in soil have a selective effect on plant populations. This results in a low diversity of species in different trophic levels (Ernst et al., 2004). Heavy metals can remain in soil for a long time. Tolerance is the capacity of plants or microorganisms to live and adapt to elevated heavy metal concentrations in soil (Dietz et al., 1999). The use of tolerant plants to extract metals from the soil for subsequent processing is both technically efficient and economically attractive (Khan et al., 2000). Studies on mechanisms of plant tolerance to heavy metal have been carried out using single metals and have been tested using simple model substrates. However, in soils contaminated by mining wastes, heavy metals such as Cu, Zn, Pb, Cd, V, Ni and Ti may be present. Cu, Zn, Pb and Cd have been shown to be toxic to plant growth (Adriano, 1986).

Soil microorganisms are important in the recovery of disturbed and potentially toxic environments because they produce plant growth stimulating substances such as hormones and vitamins, immobilize heavy metals in the soil, bind soil particles into stable aggregates which improve soil structure, reduce erosion potential and can contribute to nutrient availability to plants (Gadd, 1993;

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doi:10.1016/j.jenvman.2006.05.005

Shetty et al., 1994). The arbuscular mycorrhizal (AM) fungi are an important component of the soil microbial biomass. The symbiosis is mutualistic based on bi-directional nutrient transfer between the symbionts. The plant benefits particularly through enhanced phosphorus, water and mineral nutrient uptake (Smith and Read, 1981) which often results in better growth. The AM fungi can protect plants against the toxic effects of excessive concentrations of heavy metals (Heggo et al., 1990; Marschner, 1995). It was reported that the AM fungus *Glomus mosseae* was able to restrict Cd transfer to the plant due to Cd inmobilization by the fungi (Joner and Leyval, 1997). *Glomus deserticola* increased the resistance of plants to the presence of Cd and Pb (Arriagada et al., 2004, 2005). The fact that heavy metals reduce availability of soil nutrients to plants which are essential for their normal development and growth reinforces the importance of AM fungi for bringing these soils back into utilization (Bradshaw, 1997; Jeffries et al., 2003).

On the other hand, it is known that soil microorganisms influence the development of the AM symbiosis (Jeffries and Dodd, 1996). The saprobe fungi are important and common components of the rhizosphere. These fungi obtain greater nutritional benefit from organics and inorganic compounds released from living roots together with sloughed cells (Dix and Webster, 1995). They are also able to degrade toxic substances (Madrid et al., 2005; Wainwright, 1992). Some authors confirm the existence of synergistic effects of saprobe fungi such as *Fusarium concolor* and *Trichoderma koningii* on plant root colonization by AM fungi and on the effectiveness of AM fungi in plant resistance to heavy metals in soils (Fracchia et al., 2000; Srinath et al., 2003) especially Cd and Pb (Arriagada et al., 2004, 2005; Vogel-Mikus et al., 2005).

Plants can be used in the remediation of soils contaminated with heavy metals. In fact, plants have mechanisms for accumulation, tolerance or alleviation of high levels of heavy metals in contaminated soil (Khan et al., 2000). Many of the accumulative plants used belong to the family *Brassicaceae* which do not form AM symbiosis. However, these plants produce little biomass. Plants with higher biomass production such as trees are of more interest (Landberg and Greger, 1996). *Eucalyptus globulus* is a tree species with a high capacity to grow in impoverished or marginal soils (Montoya, 1995; Pyatt, 2001). Studies of AM fungal symbioses in trees are scarce (Wilkinson and Dickinson, 1995). *Eucalyptus* species are able to develop AM and an ectomycorrhizal symbiosis and they have a great tolerance to heavy metals (Arriagada et al., 2004; Pereira, 1998). *Glycine max* is an AM mycotrophic plant. AM fungi were able to increase *Glycine max* growth in soils contaminated with heavy metals and to decrease the concentration of heavy metals in plants (Heggo et al., 1990).

Under field conditions, different plant species live together and hyphae of AM fungi interconnect the root systems of adjacent plants changing the level of AM colonization. AM hyphae can mediate nutrient transfer between plants (Bethlenfalvay et al., 1996; Ocampo, 1986). However, the influence of intercroping on tolerance of plants to heavy metals had not been elucidated.

The aim of this work was to study the effect of saprobe and AM fungi and their interactions on the tolerance of *E. globulus* to heavy metals in soil contaminated by mining wastes, and to study the effect of *Glycine max* co-culture on this tolerance of *E. globulus*.

2. Materials and methods

2.1. Plant culture

The experiments were carried out in pots with soil contaminated by heavy metals from coal and metal mining waste disposals. The soil originated from the "Valle del Alto Guadiato" situated 70 km northwest of Cordoba province, Spain. The mining activity has been carried out during the last two centuries. The soil was a calcexerollic xerochrept type with a high Pb content (Table 1) and was sieved (2 mm mesh) before use. The land is influenced by a Mediterranean climate with a low average annual rainfall ($516\,mm\,yr^{-1}$), the annual average temperature being around 17.5 °C. The texture of the mineral soil in the experiments was mostly fine sand (3.73% clay, 7.25% silt and 89.02% sand). The soil bulk density was high, $1.66 \pm 1.78\,mg\,m^{-3}$, and it was quite permeable. The topography of the area is about 5% and disturbed soil samples were collected from different soil layers (0–40 cm in depth). Variations in the chemical composition of the contaminated soil after sieving and sterilization by steam were not observed.

Eucalyptus (*E. globulus* Labill.) and soybean (*Glycine max* L cv. Merrill) obtained from ENCE/IBERSILVA

Table 1
Chemical properties and heavy metal concentrations of soil investigated (A horizons)

pH (H_2O)[a]	5.83
Organic matter (%)	0.09
Total N (%)	0.20
P (%)	0.28
K (%)	1.31
Mg (%)	0.13
Ca (%)	13.26
Na^+ ($mg\,kg^{-1}$)	48.62
CEC ($cmol_c\,kg^{-1}$)	6.78
Fe ($mg\,kg^{-1}$)	4.93
Cu ($mg\,kg^{-1}$)	99.39
Zn ($mg\,kg^{-1}$)	186.45
Pb ($mg\,kg^{-1}$)	595.96
Cd ($mg\,kg^{-1}$)	21.48
V ($mg\,kg^{-1}$)	135.21
Ni ($mg\,kg^{-1}$)	68.27
Ti ($mg\,kg^{-1}$)	2169.50
Al ($mg\,kg^{-1}$)	236.37

[a]1:2.5 soil:water.

(Huelva, Spain) were the test plants. Seeds were surface-sterilized with $HgCl_2$ for 10 min and thoroughly rinsed with sterilized water and sown in moistened sand. After germination, 4-week old *E. globulus* and 2-week-old *Glycine max* uniform seedlings were transplanted in 1000 mL pots filled with a mixture of steam-sterilized contaminated soil:sand at a proportion of 10:1 (v:v). Plants were grown in a greenhouse with supplementary light provided by Sylvania incandescent and cool-white lamps, 400 E m^{-2} s^{-1}, 400–700 nm, with a 16/8 h day/night cycle at 25/19 °C. The temperature was controlled by a heap pump and 50% relative humidity was established and regulated by a humidifier (Defensor 505®). Plants were watered from below and fed every week with 100 mL of a nutrient solution with 50 mg L^{-1} of P (Hewitt, 1952). The dose of P was lower than recommended to avoid the negative effect of P on the AM colonization of plants.

2.2. Isolation of AM and saprobe fungi

Glomus deserticola (Trappe, Bloss and Menge) from the Instituto de Investigaciones Agrobiológicas de Galicia (CSIC) and *Glomus mosseae* (Banque European of Glomeromycota no. 12) were the AM fungi used. The AM fungal inoculum was a root-and-soil inoculum consisting of rhizosphere soil containing spores (41 spores g^{-1} of soil) and colonized root fragments (80% root length) of alfalfa (*Medicago sativa* L.) in amounts of 8 g per pot, which were predetermined to have achieved high levels of root colonization. Soil was inoculated by spreading 8 g of soil inoculum throughout each pot. Non-AM-inoculated plants were given a filtrate (Sterilized Whatman no. 1 paper) of the inoculum containing the common soil microorganisms, but free of AM fungal propagules.

The saprobe fungi *F. concolor* Schlecht. (BAFC Cult. no. 2183) (Booth, 1977) and *T. koningii* Rifai (BAFC Cult. no. F8844) (Rifai, 1969) were present in the rhizosphere soil and roots of maize cultivated in the province of Buenos Aires, Argentina. These fungi were isolated by the particle washing method using a multichamber washing apparatus (Bissett and Widden, 1972). Strains were kept at the fungal culture collection of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires in Buenos Aires, Argentina. Both saprobe fungi were transferred to tubes of potato dextrose agar (PDA, DIFCO) and 2% malt extract at 4 °C as stock culture. An aqueous suspension in sterile distilled water containing approximately 1×10^8 spores mL^{-1} of *F. concolor* and *T. koningii* was prepared from cultures grown in PDA for 1 week at 27 °C and pots were inoculated by adding 2.5 mL of this suspension to each pot (one plant per pot).

2.3. Experimental design

The experiment was under greenhouse conditions in a $3 \times 3 \times 2$ factorial completely randomized design, with five replicates. Treatments used were: (1) uninoculated controls, (2) substrate pot inoculated with *F. concolor* or *T. koningii,* (3) substrate pot inoculated with *Glomus mosseae* or *Glomus deserticola* and (4) substrate pot inoculated with *F. concolor* or *T. koningii* and either *Glomus mosseae* or *Glomus deserticola*. The plants used were: (1) *E. globulus* alone (one plant per pot) and (2) *E. globulus* + *Glycine max* (one plant of each per pot). Plants were inoculated with the AM and saprobe fungi at the time of transplanting.

2.4. Measurements and analyses

Plants were harvested 16 weeks after transplanting and dry mass was determined. After the harvest, root samples were taken from the entire root system at random, were cleared in KOH and stained with trypan blue in lactic acid (Phillips and Hayman, 1970), and the percentage of root length colonization was measured by the gridline intersect method (Giovannetti and Mosse, 1980). The shoots (leaves and stems) and roots of *E. globulus* were separated from *Glycine max* (for staining, dry weight and chlorophyll a/b measurements).

The total nitrogen (N), phosphorous (P) and potassium (K) contents in shoots of *Eucalyptus* plants were analysed as described by Mingorance (2002) using a microwave system after digestion of samples with $H_2SO_4 + H_2O_2$. Total N and P were determined by colorimetry using automatic air-segment continuous flow analysis and K was analysed by flame photometry. Cadmium (Cd) and lead (Pb) concentrations were measured after digestion of the air-dried plant samples with $HNO_3 + H_2O_2$, followed by inductively coupled plasma atomic emission spectrometry (ICP-AES), as described by Mikanova et al. (2001).

The chlorophyll a/b of *Eucalyptus* leaves was extracted with 80% (v:v) acetone at 16 weeks after transplanting and estimated by a procedure as described by Lichtenthaler (1987). The sample size for this measurement was a 5 mm diameter leaf.

2.5. Statistical analyses

Data were processed by two-way analysis of variance (ANOVA) and Fisher's protected least significant differences (LSD) when appropriate (Sokal and Rohlf, 1981) and SPSS® software was applied. Analysis by linear regression between AM root colonization and shoot dry weight, chlorophyll content and metal uptake of *Eucalyptus* was made. All data expressed as a percentage were arcsine-square-root transformed before the two-way ANOVA.

3. Results

At the end of the experiments, visual symptoms of chlorosis and necrosis in non-AM-inoculated plants were observed. Heavy metals decreased shoot dry weight of *E. globulus* when grown in the absence of AM fungi. When plants were not colonized by AM, there were no significant

Table 2
Shoot and root dry weight of *Eucalyptus globulus* grown alone or as co-culture with soybean (*Gycine max*) grown in heavy-metal-contaminated soil and inoculated or non-inoculated with *Glomus mosseae* or *G. deserticola* in presence or in absence of *Fusarium concolor* or *Trichoderma koningii*

Plants	Treatments	Shoot dry weight (g)	Root dry weight (g)	*R*/*S* ratio
E. globulus	Control	1.92a	0.51b	0.26c
	F. concolor	1.90a	0.54b	0.28c
	T. koningii	1.94a	0.57b	0.29c
	G. mosseae	2.16b	0.41a	0.18b
	G. mosseae + *F. concolor*	2.33b	0.42a	0.18b
	G. mosseae + *T. koningii*	2.34b	0.42a	0.17b
	G. deserticola	2.32b	0.43a	0.18b
	G. deserticola + *F. concolor*	2.68c	0.44a	0.16b
	G. deserticola + *T. koningii*	2.69c	0.43a	0.15b
E. globulus plus *G. max*	Control	1.86a	0.55b	0.29c
	F. concolor	1.88a	0.56b	0.29c
	T. koningii	1.90a	0.55b	0.28c
	G. mosseae	2.33b	0.43a	0.18b
	G. mosseae + *F. concolor*	2.57c	0.41a	0.15b
	G. mosseae + *T. koningii*	2.60c	0.42a	0.16b
	G. deserticola	2.89c	0.43a	0.14b
	G. deserticola + *F. concolor*	3.43d	0.41a	0.11a
	G. deserticola + *T. koningii*	3.48d	0.43a	0.12a

Column values followed by the same letter are not significantly different according to Fisher's LSD test ($P = 0.05$).

differences between the shoot dry weight of *E. globulus* cultivated alone or cultivated in the presence of *Glycine max* (Table 2). The shoot dry weight of *E. globulus* cultivated alone or in combination with *Glycine max* was increased in the presence of *Glomus mosseae* or *Glomus deserticola*. A significant correlation between the shoot dry weight of *E. globulus* and the percentage of AM root colonization was found ($r = 0.78$, $P = 0.0001$). The saprobe fungi did not affect the shoot dry weight of the *Eucalyptus* when they were inoculated individually. When plants were inoculated with the AM fungi and the saprobe fungi together, the shoot dry weight was significantly higher than when plants were inoculated only with *Glomus mosseae* or with *Glomus deserticola*. The effect of both AM and saprobe fungi on the shoot dry weight of *E. globulus* was higher when this plant was cultivated together with soybean (Table 2). The dry weight of the root of *E. globulus* was significantly lower when inoculated with *Glomus mosseae* or with *Glomus deserticola*. The joint inoculation of saprobe and AM fungi did not increase significantly the root dry weight of *E. globulus* (Table 2). The root/shoot (R/S) ratio of mycorrhizal plants was lower than that of non-mycorrhizal plants. The R/S ratio of *E. globulus* was still lower when this plant was cultivated together with soybean and inoculated with both *Glomus deserticola* and saprobe fungi (Table 2).

E. globulus reached a higher percentage of root length colonization when it was inoculated with *Glomus deserticola* than with *Glomus mosseae* (Table 3). The saprobe fungi *F. concolor* and *T. koningii* increased the AM root colonization through these AM fungi. The plants of *E. globulus* cultivated together with *Glycine max* had a higher AM root length colonization than those plants cultivated in the absence of *Glycine max* (Table 3). Heavy metals decreased the chlorophyll content of *E. globulus* non-inoculated with the AM fungi (Table 3). The results in Table 3 also show that there were no significant differences in the content of chlorophyll between the plants inoculated with the different saprobe fungi in any treatment. The AM-colonized plants had higher chlorophyll content than the non-AM-colonized plants, and a strong correlation between AM root colonization and chlorophyll content was found ($r = 0.92$, $P = 0.0001$) There were no significant differences in the chlorophyll content between *E. globulus* cultivated alone or in combination with *Glycine max*.

Table 3
Percentage of arbuscular mycorrhizal root length colonization and total chlorophyll content of *Eucalyptus globulus* grown alone or as co-culture with soybean (*Gycine max*) in heavy-metal-contaminated soil and inoculated or non-inoculated with *Glomus mosseae* or *G. deserticola* in presence or in absence of *Fusarium concolor* or *Trichoderma koningii*

Plants	Treatments	Root length colonization (%)	Total chlorophyll *a* + *b* (mg g^{-1} FW*)
E. globulus	Control	0	0.82a
	F. concolor	0	0.77a
	T. koningii	0	0.70a
	G. mosseae	40a	1.45b
	G. mosseae + *F. concolor*	50b	1.48b
	G. mosseae + *T. koningii*	51b	1.50b
	G. deserticola	50b	1.50b
	G. deserticola + *F. concolor*	64c	1.48b
	G. deserticola + *T. koningii*	72d	1.56b
E. globules plus *G. max*	Control	0	0.87a
	F. concolor	0	0.90a
	T. koningii	0	0.75a
	G. mosseae	50b	1.44b
	G. mosseae + *F. concolor*	61c	1.56b
	G. mosseae + *T. koningii*	61c	1.48b
	G. deserticola	60c	1.41b
	G. deserticola + *F. concolor*	75d	1.55b
	G. deserticola + *T. koningii*	79d	1.57b

Column values followed by the same letter are not significantly different according to Fisher's LSD test ($P = 0.05$); *: FW: fresh weight.

Heavy metals decreased the N, P and K concentrations of shoots of *E. globulus* when it it was non-inoculated with AM fungi. The N, P and K concentrations of shoots of the non-inoculated *E. globulus* with AM or saprobe fungi and cultivated alone were similar to those cultivated in the presence of *Glycine max* (Table 4). The saprobe fungi did not have a significant positive effect on the N, P and K concentrations. Both AM fungi increased the total N concentration in *E. globulus* shoots when cultivated alone or in the presence of *Glycine max* (Table 4). However, only *Glomus deserticola* increased the shoot P concentration of *E. globulus* when this plant was cultivated alone or in combination with *Glycine max* (Table 4). The concentration of K was increased only in plants of *E. globulus* inoculated with *Glomus deserticola* and cultivated in the presence of *Glycine max*.

The Cd concentration in the shoots was higher in *Eucalyptus* plants infected with *Glomus deserticola* and a significant correlation between both parameters was found ($r = 0.80$, $P = 0.0001$). The co-inoculation between *Glomus deserticola* and *T. koningii* increased Cd content in the plant shoot (Table 5). The Pb concentration in shoots was also evaluated (Table 5). Results obtained showed that the inoculation of *Glomus deserticola* enhanced the Pb concentrations in *Eucalyptus* plants and a significant correlation between both was found ($r = 0.85$, $P = 0.0001$).

Table 4
Shoot N, P and K content of *Eucalyptus globulus* grown alone or as co-culture with soybean (*Gycine max*) grown in heavy metal contaminated soil and inoculated or non-inoculated with *Glomus mosseae* or *G. deserticola* in presence or in absence of *Fusarium concolor* or *Trichoderma koningii*

Plants	Treatments	Total N (%)	P (%)	K (%)
E. globulus	Control	1.76a	0.21a	1.21a
	F. concolor	1.78a	0.23a	1.14a
	T. koningii	1.81a	0.21a	1.17a
	G. mosseae	2.96b	0.22a	1.14a
	G. mosseae+F. concolor	2.95b	0.22a	1.14a
	G. mosseae+T. koningii	3.01b	0.24a	1.16a
	G. deserticola	3.51c	0.33b	1.17a
	G. deserticola+F. concolor	3.53c	0.32b	1.17a
	G. deserticola+T. koningii	3.57c	0.35b	1.15a
E. globulus plus *G. max*	Control	1.73a	0.22a	1.17a
	F. concolor	1.79a	0.21a	1.15a
	T. koningii	1.82a	0.22a	1.15a
	G. mosseae	2.72b	0.23a	1.16a
	G. mosseae+F. concolor	2.71b	0.24a	1.19a
	G. mosseae+T. koningii	2.70b	0.24a	1.16a
	G. deserticola	3.49c	0.42c	1.52b
	G. deserticola+F. concolor	3.44c	0.42c	1.67b
	G. deserticola+T. koningii	3.53c	0.43c	1.72b

Column values followed by the same letter are not significantly different according to Fisher's LSD test ($P = 0.05$).

Table 5
Shoot Cd and Pb content of *Eucalyptus globulus* grown alone or as co-culture with soybean (*Gycine max*) grown in heavy metal contaminated soil and inoculated or non-inoculated with *Glomus mosseae* or *G. deserticola* in presence or in absence of *Fusarium concolor* or *Trichoderma koningii*

Plants	Treatments	Cd ($mg\,kg^{-1}$)	Pb ($mg\,kg^{-1}$)
E. globulus	Control	3.1a	125.4a
	F. concolor	3.2a	128.9a
	T. koningii	3.1a	130.1a
	G. mosseae	3.2a	132.3a
	G. mosseae + F. concolor	3.0a	129.4a
	G. mosseae + T. koningii	3.2a	133.5a
	G. deserticola	7.4b	284.1b
	G. deserticola + F. concolor	7.7b	293.5b
	G. deserticola + T. koningii	10.2c	301.6b
E. globulus plus *G. max*	Control	2.3a	119.3a
	F. concolor	2.4a	121.7a
	T. koningii	2.3a	123.9a
	G. mosseae	2.8a	122.2a
	G. mosseae + F. concolor	2.8a	124.5a
	G. mosseae + T. koningii	2.9a	121.8a
	G. deserticola	6.2b	274.3b
	G. deserticola + F. concolor	6.1b	276.2b
	G. deserticola + T. koningii	9.9c	281.6b

Column values followed by the same letter are not significantly different according to Fisher's LSD test ($P = 0.05$).

4. Discussion

The results of our study show that the plants of *E. globulus* developed chlorosis and necrosis when were grown in heavy-metal-contaminated soil not inoculated with AM fungi but these plants resisted the adverse soil conditions when they were inoculated with AM fungi. High amounts of heavy metals in soil can decrease plant growth and nutrient uptake (Fabig, 1982) and it is known that AM fungi protect plants against toxic actions of heavy metals (Heggo et al., 1990; Marschner, 1995). A correlation between the AM root length colonization and the shoot dry weight of *E. globulus* was found. *Glomus mosseae* and *Glomus deserticola* contributed to a better development of the plants grown in contaminated soil since they increased the total N and the quantity of chlorophyll in *E. globulus* shoots. In addition, *Glomus deserticola* increased the total P and K uptake in plant shoots. The protection of *Eucalyptus* by AM fungi against heavy metals was more evident when this plant was grown as an intercrop with soybean than as a monoculture. It has been found that the AM fungus can mediate nutrient transfer between two plants through direct hyphal connection from root to root and a competitive effect of recipient to donor plant has

been observed (Bethlenfalvay et al., 1996). The enhancement of shoot dry weight, and total P and K content of mycorrhizal *Eucalyptus* when grown together with soybean and the lack of such enhancements in non-mycorrhizal *Eucalyptus* plants suggest that there were no antagonistic or competitive effects by soybean on *Eucalyptus*. Moreover, the increase of mycorrhization of eucalyptus when grown together with soybean, which is considered a high AM mycotrophic plant (Arriagada et al., 2004; Bethlenfalvay et al., 1996), indicated that AM colonized roots of soybean may act as an additional AM inoculum source to eucalyptus root and this AM increase may also contribute to the eucalyptus growth enhancement.

The percentage of AM colonization of *E. globulus* and the beneficial action of the AM fungi was increased by the action of the saprobe fungi *F. concolor* and *T. koningii*. Several other investigations have shown such synergistic effects of these saprobe fungi on the dry weight and on heavy metal resistance of plants colonized by AM fungi (Arriagada et al., 2004; Fracchia et al., 2000; Srinath et al., 2003). The presence of the saprobe fungi *F. concolor* and *T. koningii* also contributed to the enhancement of shoot dry weight and N, P and K content of AM *Eucalyptus* grown together with soybean. However, it is not possible to determine if it was a direct beneficial action of the saprobe fungi on the AM fungi or if these saprobe fungi benefit AM fungi through their effect on soybean roots or through the modification of its root exudates (McAllister et al., 1994).

The dry weight of *Eucalyptus* roots non-inoculated with the AM fungi was higher than that of the mycorrhizal plants. The reason may be found in a finer root system which can improve the absorption of water and mineral nutrients. Less photosynthesis products were likely to be needed to maintain the mycorrhizal root system, with a consequent benefit to the plant shoot growth, which should result in lower R/S ratios (Smith and Read, 1981).

It has been found that the production of chlorophyll can significantly be reduced in plants when cultivated in soils contaminated by heavy metals (Ouzounidou, 1995). In our experiments, the total chlorophyll production increased when the plant was colonized with AM fungi and both were strongly correlated, but the chlorophyll content did not increase further when AM and saprobe fungi were inoculated together, nor when *E. globulus* was cultivated together with *Glycine max*. The higher total chlorophyll content of mycorrhizal plants apparently resulted directly in an increased photosynthetic efficiency of the plants (Gil, 1995). Earlier research suggested that some heavy metals disable the biosynthesis of chlorophyll (Ouzounidou, 1995).

Arriagada et al. (2004) observed in mycorrhizal *Eucalyptus* plants a 60% Cd uptake. The increased of Cd uptake in shoots of *Eucalyptus* when co-inoculated with *Glomus deserticola* and *T. koningii* indicates that the AM fungus–saprobe action may be species specific. Only *Glomus deserticola* may be a suitable AM fungus to remove high quantities of Cd from contaminated soils and a correlation between the level of AM colonization and the quantity of Cd in shoot of *Eucalyptus* was found. Also, the increased Pb uptake indicates a better extraction of this element from polluted soil by the AM fungus *Glomus deserticola*. In this context, Arriagada et al. (2005) also found a positive effect of *Glomus deserticola* on plant growth and a higher tolerance of the mycorrhizal plants to Pb toxicity. Higher Cd and Pb accumulation in the stem than in the leaves of eucalyptus has been observed (Arriagada et al., 2004, 2005). This redistribution of heavy metals in the less metabolically active part of the plant might explain why AM fungi increased the content of heavy metals and enhanced the growth of eucalyptus.

5. Conclusions

In conclusion, our results indicate that *E. globulus* is suitable to grow and rehabilitate heavy-metal-polluted soils when inoculated specifically with *Glomus deserticola*. Some other soil microorganisms like saprobe fungi can further improve the chances to recover these contaminated sites and bring them back into cultivation. The association of *Eucalyptus* with heavy-metal-resistant legume varieties can further help to improve the resistance of *Eucalyptus* to heavy metals. It can be assumed that such legumes will also support the nitrogen nutrition of the *Eucalyptus* provided that an effective Rhizobium–legume association can be established under such heavy-metal-polluted conditions.

Acknowledgements

Financial support of this study was provided by the Comision Interministerial de Ciencia y Tecnologia, Spain. Cesar Arriagada is thankful to the Secretaría de Estado de Educación y Universidades, Spain, for providing financial support.

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